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PHYTOTOXIC EFFECT OF AQUEOUS LEAF EXTRACT OF EUPHORBIA HIRTA L.ON SEED GERMINATION AND SEEDLING GROWTH OF SELECTED VEGETABLE CROPS

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ABSTRACT

Euphorbia hirta is a common invasive weed on the road side and waste land which may interfere with crop species due to its allelopathic effect. Aqueous extract of different concentrations (25%, 50% ,75% and 100%) of weed was used to investigate their allelopathic effect on seed germination, root length, shoot length, fresh and dry weight of some vegetable crops such as cucumber, okra and long bean. Leaf extract of E.hirta found to have inhibitory effect on germination and seedling studies. However, maximum inhibition was recorded at higher concentrations (75-100%). Hence to investigate its phytotoxic nature, and identification of allelochemical by FTIR. Results of the FTIR Spectra of the alkanes, alkenes, amides, carboxylic acids, ether, aromatic amines and organic halogen revealed the presence of different functional groups as such as N-H, -O-H, -C-H, -C=O, -C-N, -C-Cl, it could be responsible for the allelochemical properties of the weed plant E.hirta.

KEYWORDS: invasive weed, allelopathy, E. hirta, phytotoxicity.

INTRODUCTION:

One of the fascinating but controversial processes in plant ecology is the mediation of competitive interactions among plants by plant-released chemicals which can suppress the growth of other plants, a process known as allelopathy. Some weed species supplement aggressiveness by production of phytotoxic or plant growth inhibiting substances that adversely affect growth and development of other plants. Several chemicals can be released together and may exert toxicities in an additive or synergistic manner (Putnam and Tang,1986). However, there are several reports indicating that most of the allelochemicals at high concentrations are phytotoxic and show detrimental effects on plant growth and seed germination although some allelochemicals are also identified as insecticidal and pesticidal in nature. In this connection, allelopathic plants may widely be used in sustainable agriculture for their potential role in herb/weed and insect/pest management. Alleloapthic strategies aim at reducing environmental pollution and maintaining ecological balance especially soil fauna and flora through reduced use of chemical herbicides or substituting them with natural products (plant and microbial products). Allelochemicals and phytochemicals are eco-friendly and free from the problems associated with present herbicides. Hence, allelopathy is the priority area of research in the world.

Hence an attempt to study the possible allelopathic effects of commonly distributed weeds in the agricultural fields of the country (Euphorbia hirta) on germination and growth of some vegetable crops such as Ladys finger (Abelmoschus esculentus.L) Cucumber (Cucumis sativus L.) and Long bean (Vigna unguiculata)). More over, the present study was also designed to explore the preliminary phytochemical

MATERIALS AND METHODS:

All the experiment and investigations of the present study were carried out with the fully viable healthy seeds of Test crops viz., Long bean (Vigna unguiculata L.), Ladys finger (Abelmoschus esculentus .L), Cucumber (Cucumis sativus .L) were obtained from the seed store, Agriculture College, Vellayani, Thiruvananthapuram, Kerala. The donor weed species Euphorbia hiort (Euphorbiaceae), was taken into consideration for allelopathic studies and these are obnoxious weed because of their vigorous growth, easy availability and better responses towards test plant material. Fresh and healthy leaves collected from actively growing populations of weeds in various places of Thiruvananthapuram district, Kerala during the period of investigations.

The leaves of the collected weed plants were separated and air dried in shade for a week. The dried leaves were crushed into powder and stored in polythene bags for experiment. Laboratory experiments were conducted in Department of Botany ,Womens College, Thiruvananthapuram,Kerala.

Preparation of plant extracts:

10 g weed powder soaked in 100ml distilled water shaken well and it kept for 24 h. at room temperature. After 24 h, extract was filtered through cheese cloth. Different concentrations of the extracts i.e. 25%, 50% ,75% and 100% were prepared by diluting it with distilled water and the control obtained only distilled water.

Germination study:

The germination test was carried out in a sterile petridishes of 9 cm in size, placing a Whatman No. 1 filter paper on petridishes. The extract of each concentration was added to each petridish of respective treatment daily in such an amount just to allow the seed getting the favourable moisture for germination and growth. Five seeds of test plants were placed in petridish replicating 3 times. Similar volume of distilled water was used as control. The whole set of experiment was kept undisturbed at a room temperature.

The following observations were recorded on individual basis

Seed germination:

The seeds were considered as germinated when the radical emerged and the germination was recorded daily. The results were determined by counting the no. of seeds germinated. The germination percentage was calculated using the formula.

$$\text{Germination Percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seedling Growth:

After 7 days of sowing, seedlings were randomly selected and the following growth parameters were measured.

The actual length of seedling shoot and root was measured in laboratory was expressed in centimeters. And the fresh weight of seedling shoot and root was measured using an electronic balance (precision 0.001 g)

Vigour index:

The Vigour index was (VI) calculated for each replication by using the formula suggested by using Gupta et al(1996)

VI= [Root length (cm) + Shoot length] X Germination %

Extraction, Identification and Characterization of allelochemicals

Preparation of extracts:

10g portions of powdered plant materials were each separately dispersed in 100ml of each water and methanol. The solution was shaken well and left to stand at room temperature for 24hrs and was filtered with Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening using the following tests.

Phytochemical analysis:

Phytochemical screening procedures carried out were adapted from the previous work on plant analysis using the standard procedures as described by (Thenmozhi. et al, 2010.) .

IR Spectroscopic Analysis:

Infrared spectrophotometer (IR) is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of test plant (*E.hirta*) materials was considered for instrumental analysis. The powdered sample (plant specimen) was treated for IR spectroscopy (Shimadzu, IR Affinity 1, Japan).

RESULTS AND DISCUSSION:

The effects of leaf extract of Euphorbia hirta and their concentrations on seed germination of some vegetable crops are shown in (Table.1). From the experimental results, it is clear that the aqueous leaf extracts of *E.hirta*, caused significant changes in the germination percentage as compared to the control. A similar inhibitory effect caused by leaf extract of *E.hirta* on groundnut was reported by Marthama and Anitha (2012). As indicated in the table the maximum germination percentage shown in the control where no extract was used, which was 100% in okra and bean and 80% in cucumber. The highest inhibitory effect (40%) was found in okra at 75%, (60%) in long bean at 75% and 80% in cucumber at 50% concentration. The inhibitory influence of *E. hirta* may be due to the interference and possible presence of allelopathic compounds. The compounds exhibit a wide range of mechanisms of action, like inhibition of cell division, cell elongation, alteration of membrane permeability and disturbing the process of mineral uptake, water balance and photosynthesis. Interpretation of mechanism of action are complicated by the fact that individual compound can have multiple phytotoxic effects (Einhelling, 2002).

Table1. Effect of different concentration of aqueous leaf extracts of E.hirta L. on germination% of selected vegetable crops

Conc. of leaf extract	Abelmoschus esculantus	Vigna ungiculata	Cucumis sativus
Control	100	100	80%
25%	100	100	80%
50%	80	80	60%
75%	40	60	-
100%	-	-	-

The germination percentage decreases with increasing concentration of extracts and the highest inhibitory effect was shown in 100% treatment. It should be noted that E. hirta extract had significant effects on the germination of okra and long bean, where as it had strong inhibitory effect on germination of cucumber. These results suggested that E.hirta has different effects on plants species, which belong to different families With increasing concentrations of extracts the allelochemicals present in them might have acted more negatively, leading to complete inhibition of seed germination of okra, long bean and cucumber

Post germination effects on extracts:

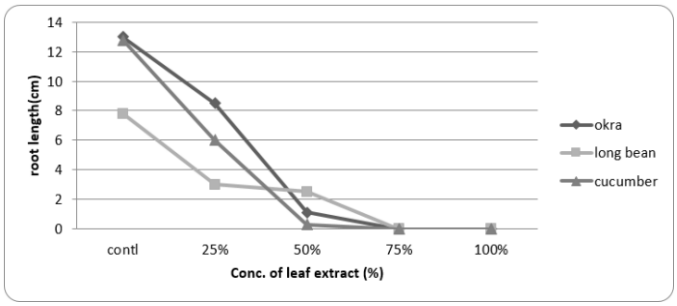
Root elongation:

Extracts of *E. hirta* leaf significantly inhibited the growth of the radicle of tested crop seeds (Fig. 1). The radicle elongation decreased by 25 and 50% leaf extracts respectively, compared to control treatment. All crop plants (okra, long bean and cucumber) did not show radicle elongation by leaf extract treatment at 100% concentration. The smallest root length was observed in all crop plants at 50% concentration. In okra (0.8), long bean (1.4cm) and cucumber (0.2cm) as compared with the control treatment 4.5, 4.0 and 6.0cm respectively. The allelopathic impact was therefore more harmful to radical (Friedman, 1995)

Shoot elongation:

The data on shoot length (cm) is presented in (Fig.1) indicated that there was significant differences among treatments. Among the crop species Abelmoschus esculentus, Vigna unguiculata, and Cucumis sativus recorded significantly lower shoot lengths (1.1, 2.5 and 0.3cm) compared to their control 13,8 and 12.8cm respectively. The maximum reduction of shoot length of all crop species was observed at 75 and 100% concentration of extracts.

The roots are more sensitive to allelochemicals than shoots. Many seedlings lost their ability to develop normally as a result of reduced radical elongation. These results also revealed that root elongation and lateral root development of crops were markedly inhibited in comparison to that of shoot elongation. The shoot is not directly coming in contact with the allelochemicals and hence its growth rate might be higher than root elongation. The different degree of inhibitory effect on root and shoot length may be due to the presence of allelochemicals in the leaf extracts of *E. hirta* are toxic which may attack a naturally occurring symbiotic relationship there by destroying the usable source of plants of a nutrients (Ayeni et al.1997).



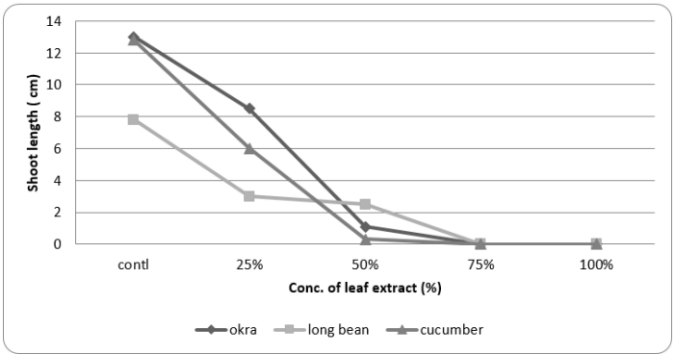
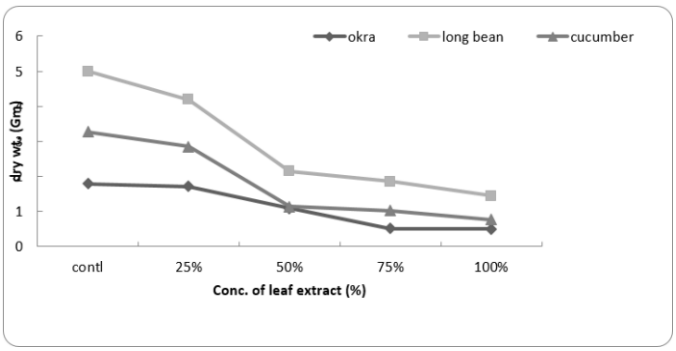


Fig. 1. Impact of aqueous leaf extracts of E. hirta L. on root and shoot length of crop plants

Fresh and dry weight of seedlings:

The extract from *E.hirta* decreases the fresh and dry weight of all test plants. The extract concentrations of 25%, 50%, 75% and 100% decreases both fresh and dry weight of given seedlings. Significant effect was noted in all the seedlings at all concentrations (Fig.2). At lower level (25%) concentration, comparatively less reduction (1.72,4.19, 2.85 g) was observed in the fresh weight of okra, long bean and cucumber seedling over control 1.79, 5.00 and 3.27 g respectively. This indicates that all the test seedlings affected by the extract can tolerate stress up to some extents, but as concentration increases, significant reduction in fresh weight and growth of seedlings occurs. Similarly, the extract from different concentrations significantly reduced the dry weight of all test seedlings over control. Maximum reduction in dry weight was noticed (0.19g) was noticed at 75% concentration in cucumber compared to okra (0.34g) and long bean (0.86g).

The adverse effect gradually increased which resulted in the reduction of dry weight in seedlings. The reason could be decreasing in fresh weight of seedlings. The allelopathic effect of Prosopsis juliflora (Sazada et al, 2009), Cassia uniflora (Ghayal et al.2011) was also reported to conclude that aqueous extract of weeds inhibited the germination of selected crops.



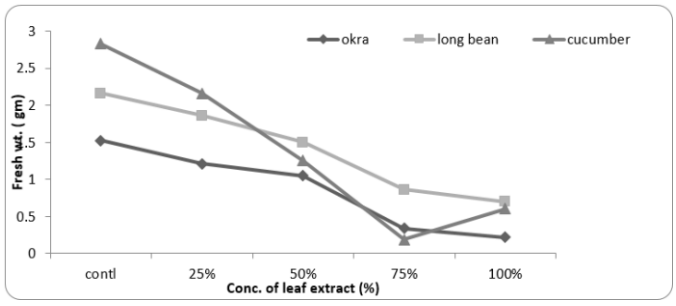


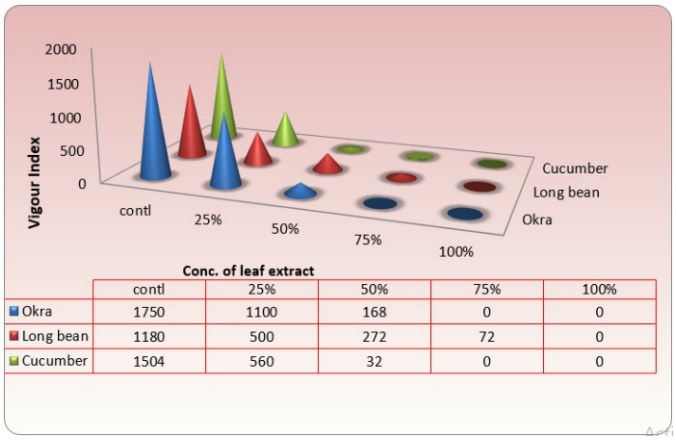
Fig. 2. Impact of aqueous leaf extracts of *E. hirta L.* on dry and shoot fresh wt. of crop plants

Vigour Index:

Seed vigor index of test crops under the influence of various concentration of extracts is presented in (Figure.3). Seedling vigor was significantly affected by the different concentrations of *E.hirta* aquous leaf extracts. These results are supported by the findings of Martha and Anitha (2012). In Ladys finger, Cucumber and Long bean, the Vigour indies observed in control was 1750, 1504 and 1180, which was reduced to 168, 32 and 272 respectively., when the seeds were treated with 50% extract, which was significantly lower than that of control. It also observed that different phytotoxicity of *E.hirta* may be attributed to the presence of variable amount of allelochemical substance in different parts that leach out under natural conditions. Foliar leachates have been regarded to be most phytotoxic in nature (Xuan et al., 2004).

Even though, in Cucurbita and long bean,the Vigour indices showed significant

reduction in all treatments from their control as shown in the subsequent. In the case of Cucurbita, a drastic decrease in Vigour index (32) was observed when the seeds were moistened with 50% concentration of leaf extracts. Seedling vigor was significantly affected by the different concentrations of *E.hirta* extracts. Channappagoudar et al. (2005) reported that the seedling vigour index in soy-bean and sunflower was significantly decreased, this indicating the allelopathic effect of the weeds. They also mentioned that the higher concentrations produced lower vigor index in all the crops. The increased inhibitory effect on germination characteristics at higher concentration of weed extract may be due to an increase in the concentration of allelochemicals. This study suggests that the allelopathic present in *E.hirta* leaves may reduce the final yield of vegetable crops in farms by inhibiting seedling growth and decreasing seed vigor. So, it is important to remove the weed from fields. The increased inhibitory effect on germination characteristics at higher concentration of weed extract may be due to an increase in the concentration of allelochemicals.



	contl	25%	50%	75%	100%
Okra	1750	1100	168	0	0
Long bean	1180	500	272	72	0
Cucumber	1504	560	32	0	0

Fig.3. Effect of leaf extract of *E.hirta* on Vigour Index of vegetable crops

Identification and charac treization of allelochjemicals:
Preliminary phytochemical analysis of the *E.hirta* leaf extract revealed the presence of active compounds such as alkalol, saponin, flavanoides, phenols, saponins, tannins, quinine, terpanoids , coumarins and cardiac glycosides are shown in Table.1. The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases. These results also demonstrated that E. hirta showed strong allelopathic potential on vegetable crops.

Table.1. Preliminary phytochemical analysis of *E.hirta* leaf extract

Sl. No.	Phytochemical Test	Reagents used (test performed)	Interference	Extracts	
				Water	Methanol
1	Alkaloid test	Wagner's reagent	Appearance of reddish brown ppt.	-	+
2	Flavonoids test	Alkaline Reagent Test	No intense yellow colour obtained	-	+
3	Phenol test	Ferric Chloride Test	Appearance of bluish black ppt.	-	+
4	Tannin test	Braymer's test	formation of yellow ppt.	+	-
5	Saponin test	Foam Test	Produce foam that lasts for more than 10 minutes	-	+
6	Terpanoids	Salkowki's test	Appearance of reddish brown ppt.	-	-
7	Quinone	Hydrochloric acid test	formation of yellow ppt.	+	-
8	Coumarine	NaOH- test	formation of yellow ppt.	-	+
9	Cardiac glycosides	Keller Kelliani test	Appearance of violet ring	-	+

'+'- present '-' absent

FTIR Spectroscopic analysis crude powder of Euphorbia hirta leaf:
The IR Spectrum (Table.2 & Fig.4) exhibited strong absorption band at 3547.09,3539.38 and 3491.16 cm^{-1} indicated the presence of N-H Stretching, heterocyclic amine. The peak at 3286.70 and 3265.49 cm^{-1} represents O-H Stretching hydroxyl group. The peak at 3209.55 and 3188.33 cm^{-1} indicated –C-H Stretching alkenes. The peak observed at 2945.30 and 2914.44 cm^{-1} show –C-H alkanes. The band at 1734.01, 1697.36 and1678.07 cm^{-1} is due to the presence of saturated C=O stretching Ketones. The peak obtained at the 1452.40 cm^{-1} representing –C-H Methylene group. The band at 1263.37 cm^{-1} indicates the presence of –C-N aromatic 1^0 amine. The peak 1029.99 cm^{-1} represents the –C-O ether. The band at 893.04, 810.10,671.23 cm^{-1} represent the presence of –C-Cl halogen.

From the IR spectroscopic analysis revealed that the presence of number of functional groups such as N-H, -O-H, -C-H, -C=O, -C-N, -C-Cl bond stretching (of alkanes, alkenes, amides, carboxylic acids, ether, aromatic amines, and organic halogen) are present in the leaf extract of *E.hirta*, it could be responsible for the allelochemical properties of the weed plant. The above result was supported by Qaisar et al.(2012). The allelochemicals present in the plant leached in the environment and cause adverse effect on receipient plants.

Table.2. IR specroscopic analysis of *E. hirta* showing characteristic absorption Peaks and Functional groups

Sl. No	Peak value	Stretching	Functional groups
1	3547.09	N-H	Heyerocyclic amine
2	3539.38	N-H	Heterocyclic amine
3	3491.16	N-H	Heterocyclic amine
4	3286.70	O-H	Hydroxyl group
5	3265.49	O-H	Hydroxyl group
6	3209.55	-CH	Alkenes
7	3188.33	-CH	Alkenes
8	2945.30	C-H	Alkanes
9	2914.44	C-H	Alkanes
10	1734.01	-C=O	Ketones
11	1697.36	-C=O	Ketones
12	1678.07	-C=O	Ketones
13	1452.40	C-H	Methylene
14	1263.37	-CN	Aromatic 1^0amine
15	1029.99	-C-O	Ether
16	893.04	-C-Cl	Halogen
17	810.10	-C-Cl	Halogen
18	671.23	-C-Cl	Halogen

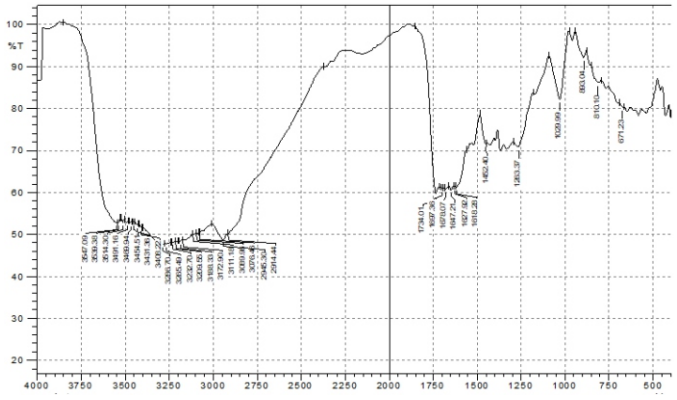


Fig. 4. FTIR Spectroscopic analysis crude powder of Euphorbia hirta leaf

CONCLUSION:
From the study it can be concluded that aqueous leaf extract of E.hirta had greater inhibitory effect on germination rate, shoot length root length, vigour index and dry weight of test crops such as okra, long bean and cucumber. The present study provides the evidence of allelopathic potential of E.hirta on the three vegetable crops namely, okra, long bean and cucumber. The allelopathic activity of plant extracts is due to the various phytotoxic compounds present in the extracts which may independently or jointly contribute to plant growth regulatory effect and inhibit germination. The effect of these weeds on the germination and seedling growth of these crops in the natural environment where additive or synergistic effects become significant even at low concentrations should also be investigated. It is also suggested that these crops should not be planted close to weeds

due to adverse effects on their growth.

The application of allelopathic compounds is an alternative weed control technique that could be incorporated into an integrated weed management programs, thereby reducing the dependence on herbicides and extending the commercial life of valuable chemicals.

Investigation is needed to isolate the individual chemical and to study their growth inhibitory and enhancing effects. There is a need for the examination of the ecological role(s) of the alleochemical in addition to its effect on plant growth. In addition, the presence of characteristic functional groups of alcohol and hydroxyl , alkane, alkenes groups, nitrogen-oxy groups, are responsible for various medicinal properties of E.hirta. In future these weed could be used as good pharmaceutical and therapeutic agents. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds for drug formulation.

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